CLAIMS

What is claimed is:

1. A method for assaying a sample for an amplification product from a target polynucleotide, comprising:

contacting the sample with an unlabelled probe polynucleotide attached to a substrate;

wherein the sample is suspected of containing the amplification product, and wherein the amplification product comprises a first label and a capture sequence;

wherein the probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least a part of the third region is complementary to at least a part of the capture sequence, and wherein the probe polynucleotide can preferentially hybridize to the amplification product and thereby disrupt formation of the stem-loop structure under at least one set of hybridization conditions;

wherein said contacting takes place under said at least one set of hybridization conditions; and

determining if the first label is associated with the substrate to determine if the amplification product is present in the sample.

2. The method of claim 1, wherein the substrate is selected from the group consisting of a microsphere, a chip, a slide, a multiwell plate, an optical fiber, and an optionally porous gel matrix.

3. The method of claim 2, wherein the substrate is a slide.

4. The method of claim 2, wherein the substrate is a first microsphere.

5. The method of claim 4, wherein the first microsphere comprises a first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics.

6. The method of claim 2, wherein the substrate is a chip.

7. The method of claim 2, wherein the substrate is a multiwell plate.

8. The method of claim 1, wherein the substrate is attached to a plurality of different unlabeled probe polynucleotides having corresponding different sequences, wherein each of said different probe polynucleotides can form a stem-loop structure, wherein each of said different probe polynucleotides can preferentially hybridize to a corresponding different amplification product and thereby disrupt formation of its stem-loop structure under said at least one set of hybridization conditions, wherein each of said corresponding different amplification products comprises a label which may be the same as or different than the first label, and determining if the label from each corresponding different amplification product is associated with the substrate.

9. The method of claim 1, wherein the amplification product is produced from an amplification process comprising a polymerase chain reaction.

10. The method of claim 1, wherein the amplification product is produced from an amplification process comprising contacting the sample with an enzyme having reverse transcriptase activity under conditions in which the enzyme can reverse transcribe RNA to DNA.

11. The method of claim 1, wherein the substrate is washed after said contacting and prior to determining if the label is associated with the substrate.

12. The method of claim 1, wherein the sample is diluted with a medium lacking the first label after said contacting and prior to determining if the label is associated with the substrate.

13. The method of claim 1, wherein the target polynucleotide has multiple alleles and the method selectively determines if an amplification product produced from a subset of the alleles is present in the sample.

14. The method of claim 13, wherein multiple amplification products are produced from said multiple alleles but the probe polynucleotide can selectively hybridize to the amplification product from only a subset of the alleles.

15. The method of claim 13, wherein the amplification product is produced from a subset of the alleles by selective amplification.

16. The method of claim 1, wherein the first label comprises an agent selected from a chromophore, a lumiphore, a fluorophore, a chromogen, a hapten, an antigen, a radioactive isotope, a magnetic particle, a metal nanoparticle, an enzyme, an antibody or binding portion or equivalent thereof, an aptamer, and one member of a binding pair.

17. The method of claim 16, wherein the agent is a fluorophore.

18. The method of claim 17, wherein the fluorophore is selected from a semiconductor nanocrystal, a fluorescent dye, a lanthanide chelate, and a green fluorescent protein.

19. The method of claim 18, wherein the fluorophore is a semiconductor nanocrystal.

20. The method of claim 19, wherein the semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, PbSe, an alloy thereof, and a mixture thereof.

21. The method of claim 20, wherein the core is CdSe.

22. The method of claim 19, wherein the semiconductor nanocrystal comprises a shell.

23. The method of claim 22, wherein the shell is ZnS.

24. The method of claim 18, wherein the fluorophore is a fluorescent dye.

25. The method of claim 24, wherein the fluorescent dye is fluorescein.

26. The method of claim 18, wherein the fluorophore is a lanthanide chelate selected from a europium chelate, a terbium chelate and a samarium chelate.

27. The method of claim 16, wherein the agent is an enzyme selected from alkaline phosphatase, horseradish peroxidase, β-galactosidase, glucose oxidase, a bacterial luciferase, an insect luciferase and sea pansy luciferase.

28. The method of claim 16, wherein the agent is selected from avidin, streptavidin, digoxigenin, and biotin.

29. The method of claim 2, wherein the first label is a fluorophore, and determining if the first label is associated with the substrate comprises:

applying a light source to the substrate that can excite the fluorophore; and

determining if a fluorescence emission from the fluorophore occurs from the substrate.

30. The method of claim 1, wherein a result of determining if the first label is associated with the probe polynucleotide is used to determine if the target polynucleotide was present in the sample prior to production of the amplification product.

31. The method of claim 1, wherein an amount of the first label associated with the probe polynucleotide is determined.

32. The method of claim 23, wherein the amount of the first label associated with the probe polynucleotide is used to determine the amount of the target polynucleotide in the sample prior to production of the amplification product.

33. The method of claim 8, wherein the hybridization of each of said different probe polynucleotides to its corresponding different amplification product can be separately determined through a different identified position at which each of said different probe polynucleotides is attached to the substrate.

34. The method of claim 8, wherein each of said different amplification products comprises a corresponding different label and wherein the hybridization of each of said different amplification products to its corresponding different probe polynucleotide can be separately determined by determining if each corresponding different label is associated with the substrate.

35. The method of claim 8, wherein the hybridization of each of said different probe polynucleotides to its corresponding different amplification product can be separately determined by the conditions under which it hybridizes.

36. The method of claim 8, wherein each different amplification product comprises a label the same as the first label.

37. The method of claim 8, wherein each different amplification product comprises a different label.

38. A method of assaying a sample for a first amplification product from a first target polynucleotide, comprising:

providing a first pair of first and second primers;

contacting the sample which is suspected of containing the first target polynucleotide with the first primer under conditions in which the first primer can hybridize to the target polynucleotide and can be extended to form a first primer extension product;

altering the sample conditions to allow dissociation of the first primer extension product from the first target polynucleotide;

contacting the sample with the second primer under conditions in which the second primer can hybridize to the first primer extension product and be extended to form a second primer extension product, wherein the second primer is complementary at its 3' end to the first primer extension product at a position in the first primer extension product which is 3' to the first primer;

wherein one of the first and second primer extension products thus formed is the first amplification product and comprises a first capture sequence and a first label;

altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product;

contacting the sample with a first probe polynucleotide attached to a substrate under hybridization conditions in which the first probe polynucleotide can hybridize to the first amplification product;

wherein the first probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the first probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least a part of the third region is complementary to at least a part of the first capture sequence, and wherein the first probe polynucleotide preferentially hybridizes to the first amplification product to thereby disrupt formation of the stem-loop structure under the hybridization conditions; and

determining if the first label is associated with the first probe polynucleotide.

39. The method of claim 38, wherein the first target polynucleotide is DNA.

40. The method of claim 38, wherein the first target polynucleotide is RNA.

41. The method of claim 40, wherein an enzyme having reverse transcriptase activity is used to form the first primer extension product from the first target polynucleotide.

42. The method of claim 38, wherein the first target polynucleotide is single-stranded.

43. The method of claim 38, wherein the first target polynucleotide is double-stranded.

44. The method of claim 38, wherein the sample is again contacted with the first and second primers after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product to form a plurality of first and second primer extension products.

45. The method of claim 38, wherein altering the sample conditions to allow dissociation of the first primer extension product from the target polynucleotide comprises heating the sample.

46. The method of claim 38, wherein altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product comprises heating the sample.

47. The method of claim 38, further comprising concurrently assaying the same sample for a second amplification product from a second target polynucleotide, comprising, in the same steps:

providing a second pair of third and fourth primers;

contacting the sample which is suspected of containing the second target polynucleotide with the third primer under conditions in which the third primer can hybridize to the second target polynucleotide and can be extended to form a third primer extension product;

altering the sample conditions to allow dissociation of the third primer extension product from the second target polynucleotide;

contacting the sample with the fourth primer under conditions in which the fourth primer can hybridize to the third primer extension product and be extended to form a fourth primer extension product, wherein the fourth primer is complementary at its 3' end to the third primer

extension product at a position in the third primer extension product which is 3' to the third primer;

wherein one of the third and fourth primer extension products thus formed is the second amplification product and comprises a second capture sequence and a second label which may be the same as or different than the first label;

altering the sample conditions to allow dissociation of the fourth primer extension product from the third primer extension product;

contacting the sample with a second probe polynucleotide attached to a substrate, which may be the same as or different than the substrate to which the first probe polynucleotide is attached, under hybridization conditions in which the second probe polynucleotide can hybridize to the second amplification product;

wherein the second probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the second probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least a part of the third region of the second probe polynucleotide is complementary to at least a part of the second capture sequence, and wherein the second probe polynucleotide preferentially hybridizes to the second amplification product to thereby disrupt formation of the stem-loop structure under the hybridization conditions; and

determining if the second label is associated with the second probe polynucleotide.

48. The method of claim 47, wherein the first and second amplification products are produced from a single locus.

49. The method of claim 48, wherein the first and second amplification products differ by a single nucleotide.

50. An amplification product assay complex comprising a substrate comprising an unlabelled probe polynucleotide hybridized to an amplification product from a target polynucleotide,

wherein the amplification product comprises a capture sequence and a label,

wherein the probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop,

wherein at least a part of the third region is hybridized to at least a part of the capture sequence, and wherein the stem-loop structure is not formed as a result of the probe polynucleotide being hybridized to the amplification product.

51. A method of forming an amplification product assay complex, comprising:

hybridizing the amplification product to an unlabelled probe polynucleotide attached to a substrate under a first set of hybridization conditions to form an amplification product assay complex;

wherein the amplification product comprises a first label and a first single-stranded capture sequence;

wherein the probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least a part of the third region is complementary to at least a part of the capture sequence, and wherein the probe polynucleotide hybridizes to the amplification product and thereby disrupts formation of the stem-loop structure under the first set of hybridization conditions.

52. An amplification product assay array comprising a plurality of different unlabelled probe polynucleotides attached to a substrate, wherein each of said different unlabelled probe polynucleotides is attached at an identifiable location on the substrate, wherein each of said different probe polynucleotides can preferentially hybridize to a corresponding different amplification product, each of said corresponding different amplification products comprising a label that can be the same or different as the label on the other different amplification products,

wherein each of said different probe polynucleotides comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein each of said different probe polynucleotides can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop;

wherein at least a part of the third region of each of said different probe polynucleotides is complementary to at least a part of its corresponding different amplification product, and wherein each of said different probe polynucleotides can preferentially hybridize to its corresponding different amplification product and thereby disrupt formation of its stem-loop structure under at least one set of hybridization conditions.

53. A kit comprising:

a substrate attached to an unlabeled probe polynucleotide comprising first and second complementary regions and a third region located between the first and second complementary regions, wherein the probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop, wherein at least a part of the third region is complementary to at least a part of a capture sequence of an amplification product from a target polynucleotide, wherein the unlabeled probe polynucleotide can preferentially hybridize to the amplification product and thereby disrupt formation of the stem-loop structure under at least one set of hybridization conditions;

a reagent for incorporating a label into the amplification product;

a housing for retaining the substrate and the reagent; and

instructions provided with said housing that describe how to use the components of the kit to assay a sample for the amplification product.

54. The kit of claim 53, wherein the reagent is a labeled nucleotide.

55. The kit of claim 53, wherein the reagent is a labeled primer.

56. The kit of claim 53, wherein the substrate is attached to a plurality of different unlabelled probe polynucleotides, wherein each of said different unlabelled probe polynucleotides is attached at an identifiable location on the substrate, wherein each of said different probe polynucleotides can preferentially hybridize to a corresponding different amplification product, each of said corresponding different amplification products comprising a label that can be the same or different as the label on the other corresponding different amplification products, wherein each of said different probe polynucleotides comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein each of said different probe polynucleotides can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop, wherein at least a part of the third region of each of said different probe polynucleotides is complementary to at least a part of its corresponding different amplification product, and wherein each of said different probe polynucleotides can preferentially hybridize to its corresponding different amplification product and thereby disrupt formation of its stem-loop structure under at least one set of hybridization conditions, and

wherein said instructions further describe how to use the components of the kit to assay the sample for each of said corresponding different amplification products.

57. An article of manufacture, comprising:

a substrate attached to an unlabeled probe polynucleotide;

wherein the probe polynucleotide comprises first and second complementary regions and a third region located between the first and second complementary regions, and further wherein the probe polynucleotide can form a stem-loop structure in which the first and second complementary regions hybridize to each other to form a stem and the third region forms a loop.

58. The article of claim 57, wherein a plurality of different unlabeled probe polynucleotides are attached to the substrate, each of said different unlabeled probe polynucleotides able to form a stem-loop structure and having a different sequence.